Averrhoa carambola L., *Syzygium cumini* (L.) Skeels and *Cissus sicyoides* L.: medicinal herbal tea effects on vegetal and animal test systems

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ABSTRACT. Since folk medicine has been greatly appreciated for centuries, many researchers decided to study more deeply the curative qualities of plants. In the present study, meristematic cells of *Allium cepa* L. were used as vegetal test system and bone marrow cells of Wistar rats as animal test system. Both were treated *in vivo* to evaluate whether the plants *Averrhoa carambola* L., *Syzygium cumini* (L.) Skeels and *Cissus sicyoides* L. presented cytotoxic and mutagenic effects and whether they resulted in cell alterations in their morphology, chromosomes or cell cycle division. Herbal teas were prepared as normally done by the population, albeit in two different concentrations, the usual concentration and a concentration ten times higher. Rats were treated with only one concentration of teas. Results showed that teas did not alter the cell cycle of *Allium cepa* L., with the exception of the 24 hours analysis after suspension of treatment (recovery of treatments), with a lower concentration of *Averrhoa carambola*. The latter had a low mitotic index when compared to control and to the post-treatment analysis, showing an inhibition of cell division. The three herbal teas neither induced an increase in the number of chromosomal damage in bone marrow cells of Wistar rats nor altered the cell division cycle. Results are important in so far as these plants are used as therapeutic agents.

Key words: medicinal herbs, chromosome damage, mutagenicity test.

RESUMO. *Averrhoa carambola* L., *Syzygium cumini* (L.) Skeels e *Cissus sicyoides* L.: efeitos dos chás de plantas medicinais sobre os sistemas-teste vegetal e animal. As plantas medicinais têm sido muito estudadas devido aos seus efeitos curativos. Neste estudo foram utilizados o sistema teste vegetal em células meristemáticas de *Allium cepa* L. e o sistema teste animal em células da medula óssea de ratos Wistar tratados *in vivo* para avaliação dos efeitos citotóxicos e mutagênicos das plantas *Averrhoa carambola* L., *Syzygium cumini* (L.) Skeels e *Cissus sicyoides* L., analisando-se o ciclo de divisão celular, morfologia e cromossomos. Os chás das plantas foram preparados da maneira usada pela população e em duas diferentes concentrações, a usual e outra dez vezes maior. Os chás foram aplicados em doses únicas nos ratos. A avaliação demonstrou que os chás não alteram o ciclo celular de *Allium cepa* L., exceto na análise 24 horas após a retirada do tratamento (recuperação dos tratamentos) com a menor concentração de *Averrhoa carambola* L., o qual apresentou um baixo índice mitótico quando comparado ao controle e à análise imediatamente após o tratamento, mostrando uma inibição da divisão celular. Os três chás não induziram aumento do número de alterações cromossômicas em células da medula óssea de ratos Wistar e não alteraram o ciclo de divisão celular. Os resultados são importantes pelo fato de que essas plantas são usadas como agentes terapêuticos pela população.

Palavras-chave: plantas medicinais, aberração cromossômica, teste de mutagenicidade.

Medicinal herbs have been popularly used in tea form all over the world, although nearly all of them have never been scientifically tested. The teas and plant infusions may have substances with toxic or even mutagenic effects. On the another hand, the consumption of teas can suppress the effects of certain powerful mutagenic agents in human beings. *Averrhoa carambola* L., *Syzygium cumini* (L.) Skeels and *Cissus sicyoides* L. are examples of medicinal plants used as teas and whose cytotoxic and mutagenic effects are unknown.

The star fruit tree (*Averrhoa carambola*) is a small, shrub-like ornamental tree with eatable fruit, rich in vitamins, phosphorus and oxalic acid. The plant belongs to the Oxalidaceae family and is native to India. The star fruit has an antidiabetic and high blood tension lowering-effect, an appetite stimulator, and an anti-diarrhea, anti-scurvy, antipyretic attributes (Moreira, 1985). It is used topically on poisonous bites and stings (Iamoni, 1997), and its fruit is prescribed against eczemas. It is a diuretic and combats kidney and bladder diseases.

Syzygium cumini is a big shrub from the Myrtaceae family, native to southern Asia. The bark of the tree is used against dysentery, hemorrhage and leukorrhea (Moreira, 1985). It is also used to treat noninsulin-dependent type II diabetes, because it lowers the blood glucose level to normal (Moreira, 1985; Conceição, 1987; Iamoni, 1997). It has also a diuretic effect (Silva-Netto *et al*., 1987). It is used to treat diarrhea and infections from the upper-respiratory-tract since it has an antimicrobial property (Corrêa *et al*., 1998). Its chemical composition consists of tannins, resins (gambol), terpans (α-pigeon, β-pigeon, limonene), acids (gallic, palmitic, stearic, oleic), steroids (phytosterol), saponinic glycosides (antimelin) and flavanols (Albuquerque, 1989; Corrêa *et al*., 1998).

Cissus sicyoides belongs to the Vitaceae family. It is used against rheumatism, abscesses, muscle inflammation, epilepsy, stroke and convulsions. Also, it is a hypotensor and bloodstream activator, and causes deep sleep and perspiration (Santana, 1984). Its leaves are largely used for treating diabetes and are known as "similar to insulin" because of their hypoglycemic effect (Martins *et al*., 1995). The tea is diuretic and good for the kidney. It expels impurities and also stones from the bladder and kidney. It also balances the blood pressure and ensures healing of wounds. Some of its components are α- and β-carotenes.

To evaluate the cytotoxic and mutagenic effects of these three herbal teas the meristematic cells of *Allium cepa* L. root were used as vegetal test system; bone marrow cells of Wistar rats were used as animal test system; both were treated *in vivo*. Our aim was to evaluate whether the plants caused morphological cell alterations, as chromosome aberrations and in the cellular division cycle.

Material and methods

Medicinal herbs. *Averrhoa carambola*, *Syzygium cumini* and *Cissus sicyoides* were obtained from the Irenice Silva Medicinal Herb Garden of the State University of Maringá. The infusions were prepared in the same way as usually done by the population, by adding boiling water to the leaves *in natura* and then leaving them to stand for cooling before straining. Teas were prepared in two different concentrations, one corresponding to that normally used by the population (0.07 mg/ml) and another ten times higher (0.70 mg/ml).

***Allium cepa* root-tip cells.** The bulbs were placed in flasks with aerated water to root at room temperature. We considered the control group as 0 hour until the first root sample was obtained to serve as a control of the bulb (Co). These were then placed for 24h either into the three plant teas prepared in the two different concentrations or into the water in the control group. Next, some more roots were removed (Tr) and the bulbs returned to water for 24h to observe whether there was any recovery (Re) from possible damage. Roots were fixed and stained with Feulgen reaction and mounted on permanent slides.

The slide analysis was done in a "blind" test under a 40x lens optical microscope. One thousand cells per bulb were analyzed, adding up 6,000 per control, treatment and respective recovery. Cells with morphological structural alterations were analyzed and the Mitotic Index (MI) determined. Statistical analysis was done by the Chi-square test ($\alpha = 0.05$).

Bone marrow cells of Wistar rat. Wistar rats, *Rattus norvegicus*, were obtained from the Central Vivarium of the Maringá State University, weighing approximately 100 g (b.w. - body weight). Three males and three females were used for both the control and the treatment groups. Rats were put down 24h by intraperitoneal injection, after only treatment with 1ml of the tea solutions with the two different concentrations (0.07 mg/ml and 0.7 mg/ml) of the three plants.

One hour and a half before being killed, a dose of 0.5 ml/100g b.w. of colchicine 0.16% was injected in the animals. Bone marrow cells were obtained according to Ford and Hamerton (1956), with some modifications. Chromosomal analysis of the slides was done under a 100x immersion lens optical microscope. One hundred metaphases per animal were analyzed in a "blind" test, adding up 600 metaphases per control and treatment group. For each sex, the MI was calculated for the 5,000 cells, adding up 10,000 cells per group. The statistical analysis was done by the Chi-square test ($\alpha = 0.05$). The animal positive controls were treated with 1.5 mg cyclophosphamide (CP)/100 g body weight.

Results

***Allium cepa* root-tip cells.** Table 1 shows the results of total mean of the Mitotic Index (MI), total number of analyzed cells and the cell number at the different cell cycle phases (interphase, prophase, metaphase, anaphase and telophase). They were obtained for each group of six onions: the control-control, the control and the treatment, with the teas from *Averrhoa carambola* L., *Syzygium cumini* and *Cissus sicyoides* plants, with the two different concentrations and respective recoveries.

Total chi-square was calculated for the mitotic total mean indexes between the controls and respective treatments, between the treatments and respective recoveries and between controls and respective recoveries and, also, between these results and the ones obtained for the control of the control at the respective times for sampling. Only the result obtained between the control at 0.07 mg/ml concentration of *Averrhoa carambola* and its respective recovery (χ^2 = 6.21, α = 0.05) was statistically significant. After treatment with 0.07 mg/ml concentration of *Averrhoa carambola* tea, the root's meristematic cells of the six bulbs did not respond to the recovery process, showing a mitotic total mean index of 2.3. This is equivalent to half the treatment's mitotic index (5.6) and a quarter of the control's mitotic index (10.3). It seems that this inhibitory effect of the treatment was cumulative because there was a maintenance or even an increase of this effect after the recovery time in water. Such significant result was repeated when this recovery result was compared to the one obtained at 48h of the control in water (MI=8.0 e χ^2 = 4.06, α = 0.05), confirming the lack of recovery after this treatment. In the remaining treatments with the three plants and the control and in spite of the decrease in the mitotic index after 24h which have been maintained, in most cases, after 24h of recovery in water, these differences in the mitotic total mean index were not statistically significant (α = 0.05). This fact shows that neither the treatments nor the recoveries altered the cell cycle when it is compared to controls.

Bone marrow cells of Wistar rats. Table 2 shows the results of total mean of MI, the total metaphases analyzed and the number of alterations obtained from male and female Wistar rats, non-treated control and treated with the *Averrhoa carambola*, *Syzygium cumini* and *Cissus sicyoides* teas, using the two different concentrations during the acute treatment.

Table 1. Total cells analyzed, total number of cells in different phases of the cell cycle (I, P, M, A and T) and total mean of Mitotic Index (MI), obtained from different groups in *Allium cepa* L. root–tip cells, control-0h (Co), treatments-24h (Tr) and respectives recoveries-24h (Re) with the two different concentrations of *Averrhoa carambola* (*Ac*), *Syzygium cumini* (*Sj*) and *Cissus sicyoides* (*Cs*)

Treatment	Groups	Cell	MI	Number of Cells				
(mg/ml)		Total	%	I	P	M	A	T
	Co	6000	14.1	5157	456	155	154	78
Control	Tr	6000	11.6	5307	360	123	151	59
	Re	6000	8.0	5518	267	114	68	33
	Co	6000	10.3	5385	341	135	91	48
Ac (0.07)	Tr	6000	5.6	5662	197	76	47	18
	Re	6000	2.3⋆	5861	82	23	25	09
	Co	6000	8.7	5478	326	97	68	31
Ac (0.70)	Tr	6000	6.6	5602	215	103	56	24
	Re	6000	8.3	5504	274	103	86	33
	Co	6000	10.5	5371	376	107	95	51
Sj (0.07)	Tr	6000	6.8	5593	235	81	59	32
	Re	6000	8.6	5486	279	105	91	39
	Co	6000	8.2	5507	279	113	56	45
Sj (0.70)	Tr	6000	7.0	5579	263	96	46	16
	Re	6000	6.8	5594	211	103	59	33
	Co	6000	8.7	5479	330	92	50	49
Cs (0.07)	Tr	6000	8.6	5483	261	132	79	45
	Re	6000	8.0	5521	268	93	76	42
	Co	6000	10.4	5376	400	105	76	43
Cs (0.70)	Tr	6000	9.2	5451	306	121	78	44
	Re	6000	8.9	5468	317	118	58	39

I: Interphase, P: Prophase, M: Metaphase, A: Anaphase and T: Telophase; ⋆ statistically significant

Table 2. Total mean of Mitotic Index (MI), total and types of alterations and total metaphases analyzed from Wistar rats, control (Co), positive control (CP) and treated (acute treatment) with two different concentrations of *Averrhoa carambola* (*Ac*), *Syzygium cumini* (*Sj*) and *Cissus sicyoides* (*Cs*)

Treatments	MI	Total number of the cells with alteration (%)	Number of types chromosomal alterations				Total number of metaphase analyzed
			Gap		Break		
(mg/ml)	%		ct	cr	ct	cr	
Control	1.32	2 (0.3)	0	1	1	0	600
CP	1.32	79 (13.2)⋆	6	0	46	27	600
Ac (0.07)	1.55	1 (0.2)	0	1	0	0	600
(0.70)	1.83	0	0	0	0	0	600
Sj (0.07)	1.51	1 (0.2)	1	0	0	0	600
(0.70)	2.21	0	0	0	0	0	600
Cs (0.07)	2.28	1 (0.2)	1	0	0	0	600
(0.70)	2.49	1 (0.2)	0	0	0	1	600

ct: chromatidic; cr: chromosomal; CP - 1.5 mg cyclophosphamide/100g body weight. ⋆ statistically significant

Chi-square test (α= 0.05) showed that *Averrhoa carambola Syzygium cumini* and *Cissus sicyoides* teas at 0.07 and 0.70 mg/mL did not induce a statistically significant increase in the number of the cell with chromosome alterations in bone marrow cells of Wistar rats. Compared to the results obtained for the

non-treated controls, there was no alteration in the cell division index after the treatments.

Discussion

Data show that teas from *Averrhoa carambola*, *Syzygium cumini* and *Cissus sicyoides*, tested in concentrations used by the population and in concentrations ten times higher than usual in a twenty-four hour treatment, caused no alteration in the cellular cycle of meristematic cells of *Allium cepa* L. (except in the recovery group treated with water during 24h after 0.07 mg/mL of *Ac* tea). The *Allium cepa* L. test system is well accepted for the study of cytotoxic effects, because its roots are in direct contact with the tested substance, allowing the evaluation of different concentrations at different times of treatment. Chromosomal alterations and alterations of the meristematic cells' cycle division of the onion root have been frequently used to warn the population about the consumption of the product. Current research presents no indication against the ingestion of the teas of the three analyzed plants. This observation seems to be corroborated by the results obtained in the animal test system.

Recent studies show that the risk of cancer formation has been reduced with the consumption of green tea (Gao *et al*., 1990). Among the beneficial effects of this tea are inhibition of carcinogenesis and mutagenesis (Wang *et al*., 1991, 1992; Yamane *et al*., 1991; Yang and Wang, 1993, Yamada and Tomita, 1994); prevention of arteriosclerosis (Kono *et al*., 1992, Stampfer and Rimm, 1993); reduction of serum cholesterol (Kono *et al*., 1992; Stensvold *et al*., 1992) and inhibition of nitrosamine formation in nitrosation reactions (Jain *et al*., 1989; Stich, 1992). Most of these effects have been attributed to the antioxidative and free-radical scavenging properties of tea, particularly of polyphenolic compounds (Yang and Wang, 1993; Ho *et al*., 1992; Klaunig, 1992, Stavric *et al*., 1996). Several studies show that the antimutagenic and anticarcinogenic effects of green tea are due to the presence of tea polyphenols and catechins, especially epigallocatechin gallate (Yang and Wang, 1993, Fujiki *et al*., 1992, Mukhtar *et al*., 1994). Using rat erythroblastic leukemic cells, Fujie *et al.* (1993) verified that crude catechins extracted from green tea suppressed sister-chromatid exchanges induced by trihalomethanes, formed in the chlorination process of water. The aqueous extract from green tea showed a powerful antimutagenic effect in routinely used concentrations in human's daily diet against the major classes of occupational carcinogens (Bu-Abbas *et al*., 1994a). In another work Bu-Abbas *et al.* (1994b) evaluated the selective induction of certain hepatic proteins and peroxissomic proliferation by the green tea.

Several authors have reported the antimutagenic effect of black tea extract by various compounds such as aflatoxin B1, benzo[*a*]pyrene, nitrate derivatives and, most recently, the heterocyclic aromatic amines (Ito *et al*., 1989; Jain *et al*., 1989, Wang *et al*., 1989; Ho *et al*., 1992, Yamada and Tomita, 1994; Yen and Chen, 1994; Apostolides *et al*., 1996). This latter compound is produced in high-temperature cooking during meat preparation. It has a powerful mutagenic and carcinogenic activity in animals (Stavric *et al*., 1996, Felton *et al*., 1992). Black and green tea extracts inhibited the mutagenicity of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine, formed during cooking of high protein foods (Apostolides and Weisburger, 1995). In a study undertaken by Shiraki *et al.* (1994) using polyphenols and theaflavins of black tea, the authors observed that the theaflavins scavenge free radicals, producing antimutagenic and antioxidative effects *in vitro* in low concentrations. The authors suggested that these compounds could have a positive role in our daily lives as a prevention of several diseases, cancers and aging, for which lipid peroxides or active oxygen are relevant.

The suppressant effect of the crude extract of three kinds of teas was evaluated for chromosomal aberrations present in man's daily diet, in the presence of fraction S9, together with benzo[*a*]pyrene or mitomycin C, in mice and Chinese hamster ovary cells (CHO) cultures: the green tea of Japan, the Po-lei tea of China and the Rooibos tea of South Africa. Studies suggest that in some cases the catechins found in some samples had an antimutagenic effect (Sasaki *et al*., 1993). Confirming the previously obtained results in *in vitro* test system, Horikawa *et al.* (1994) treated male mice subcutaneously with benzo[*a*]pyrene. Mice received concomitantly an aqueous extract of six Chinese medicinal teas for 50 weeks. The authors verified that the antimutagenicity of the isolated fractions in that study could be due to the active tannins. The antimutagenic properties of soluble instant tea were evaluated by Constable *et al.* (1996) using the Ames test. Results obtained in this study suggest that the catechins are not the sole compounds responsible for the protective and antioxidant effects of teas. The authors discuss the oxidation that occurs during tea processing, polymerizing the catechin monomers and forming other compounds.

Bu-Abbas *et al.* (1996) compared the antimutagenic effect of green, black and decaffeinated teas and concluded that flavanols are the major tea components responsible for this activity.

The chemioprotective benzo[*a*]pyrene on human lymphocytes treated with Purnark, a mixture of solvent extracts of natural products, was reported by Ghaisas and Bhide (1994). There is a confirmation on the antimutagenic effect of alcoholic extract of lemon grass plant (*Cymbopogon citratus* Stapf), commonly used in the tea form in diets and in medicine, over many known mutagens in *Salmonella typhimurium* (Vinitketkumnuen *et al*., 1994). Nakamura *et al.* (1997) evaluated the suppressant effects of clastogenicity of Tochu tea, an aqueous extract obtained from the leaves of *Eucommia ulmoides*, a popular beverage in Japan, in CHO and mice.

Among the plants tested in this work, only the species *Syzygium cumini* and *Cissus sicyoides* have some known chemical compounds. The tannins, gallic acid and flavanols in *Syzygium jambolanum* might be responsible for the non cytotoxicity and mutagenicity verified in the vegetal and animal tested systems. The β-carotene compound found in *Cissus secioidea* had a radioprotective effect in splenocytes, reticulocytes and spermatids of mice exposed to X-rays (Salvadori *et al*., 1996). It had an important role in reducing mutagenicity of many chemicals (Salvadori *et al*., 1992a, b, 1993, 1994).

Additional tests using chronic exposition, as humans do, must be undertaken to confirm the non-mutagenic effect of the teas from *Averrhoa carambola Syzygium cumini* and *Cissus sicyoides*. The important fact in this research is that in the conditions of the experiment, there is no indicative of the mutagenic effects of the compounds found in beverages consumed by the population. This is important when one considers the fact that these medicinal teas are the only alternative for the low-income community, deprived of any medical assistance, to improve their health.

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References

ALBUQUERQUE, J.M. *Plantas medicinais de uso popular*. Brasília: Associação Brasileira de Educação Agrícola Superior, 1989.

APOSTOLIDES, Z.; WEISBURGER, J.H. Screening of tea clones for inhibition of PhIP mutagenicity. *Mutat. Res.*, Amsterdam, v. 326, p. 219-225, 1995.

APOSTOLIDES, Z. *et al*. Inhibition of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) Mutagenicity by Black and Green Tea Extracts and Polyphenols. *Mutat. Res.*, Amsterdam, v. 359, p. 159-163, 1996.

BU-ABBAS, A. *et al*. Marked antimutagenic potential of aqueous green tea extracts: mechanism of action. *Mutagenesis*, Oxford, v. 9, p. 325-331, 1994a.

BU-ABBAS, A. *et al*. Selective induction of rat hepatic CYP1 and CYP4 proteins and of peroxisomal proliferation by green tea. *Carcinogenesis*, Oxford, v. 15, p. 2575-2579, 1994b.

BU-ABBAS, A. *et al*. A Comparison of the antimutagenic potential of green, black and decaffeinated teas: contribution of flavanols to the antimutagenic effect. *Mutagenesis*, Oxford, v. 11, p. 597-603, 1996.

CONCEIÇÃO, M. *Plantas medicinais do ano 2000*. Brasil: Thesaurus Ed., 1987.

CONSTABLE, A. *et al*. Antimutagenicity and catechin content of soluble instant teas. *Mutagenesis*, Oxford, v. 11, p. 189-194, 1996.

CORRÊA, A.D. *et al. Plantas medicinais: do cultivo à terapêutica*. Petrópolis: Editora Vozes, 1998.

FELTON, J.S. *et al.* Chemical analysis, prevention, and low-level dosimetry of heterocyclic amines from cooked food. *Cancer Res.*, Philadelphia, v. 52, p. 2103-2107, 1992.

FORD, C.E.; HAMERTON, J.L. A colchicine, hypotonic citrate, squash sequence for mammalian chromosome. *Stain Technol.*, Jiloviste-Stenady, v. 31, p. 247-251, 1956.

FUJIE, K. *et al*. Sister-chromatid exchanges induced by trihalomethanes in rat erythroblastic cells and their suppression by crude catechin extracted from green tea. *Mutat. Res.*, Amsterdam, v. 300, p. 241-246, 1993.

FUJIKI, H. *et al*. Anticarcinogenic effects of (-) Epigallocatechin Gallate. *Prev. Med.*, Orlando, v. 21, p. 503-509, 1992.

GAO, G.D. *et al*. Initial study of antitumorigenesis of green tea (animal test and flow cytometry). *Tumori*, Roma, v. 10, p. 42-44, 1990.

GHAISAS, S.D.; BHIDE, S.V. In vitro studies on chemoprotective effect of purnark against benzo(*a*)pyrene-induced chromosomal damage in human lymphocytes. *Cell Biol. Int.*, London, v. 18, p. 21-27, 1994.

HO, C.-T. *et al*. Antioxidative effect of polyphenol extract prepared from various chinese teas. *Prev. Med.*, Orlando, n. 21, p. 520-525, 1992.

HORIKAWA, K. *et al*. Moderate inhibition of mutagenicity and carcinogenicity of benzo[*a*]pyrene, 1,6-dinitropyrene and 3,9-dinitrofluoranthene by chinese medicinal herbs. *Mutagenesis*, Oxford, n. 9, p. 523-526, 1994.

IAMONI, R. *A cura pelos remédios caseiros.* Rio de Janeiro: Ediouro, 1997.

ITO, Y. *et al.* Chromosome aberrations induced by aflatoxin b_1 in rat bone marrow cells *in vivo* and their

suppression by green tea. *Mutat. Res.*, Amsterdam, v. 222, p. 253-261, 1989.

JAIN, A.K. *et al*. Crude tea extracts decrease the mutagenic activity of *n*-methyl-n'-nitro-n-nitrosoguanidine in vitro and in intragastric tract of rats. *Mutat. Res.*, Amsterdam, v. 210, p. 1-8, 1989.

KLAUNIG, J.E. Chemopreventive effects of green tea components on hepatic carcinogenesis. *Preventive Medicine*, Orlando, v. 21, p. 510-519, 1992.

KONO, S. *et al*. Green tea consumption and serum lipid profiles: a cross-sectional study in Northern Kyusshy, Japan. *Prev. Med.*, Orlando, v. 21, p. 526-531, 1992.

MARTINS, E.R. *et al*. *Plantas medicinais.* Viçosa: Imprensa Universitária, 1995.

MOREIRA, F. *As Plantas que curam.* Brasil: Hemus Ed., 1985.

MUKHTAR, H. *et al.* Green tea and skin-anticarcinogenic effects. *J. of Investig. Dermatol.*, Malden, v. 102, p. 3-7, 1994.

NAKAMURA, T. *et al*. Antimutagenicity of tochu tea (an aqueous extract of *Eucommia ulmoides* Leaves): 1. the clastogen-suppressing effects of tochu tea in cho cells and mice. *Mutat. Res.*, Amsterdam, v. 388, p. 7-20, 1997.

SALVADORI, D.M.F. *et al*. The protective effect of β-carotene on genotoxicity induced by cyclophosphamide. *Mutat. Res.*, Amsterdam, v. 265, p. 237-244, 1992a.

SALVADORI, D.M.F. *et al*. Beta-carotene as a modulator of chromosomal aberrations induced in mouse bone marrow cells. *Environmental Molecular Mutagenesis*, v. 20, p. 206-210, 1992b.

SALVADORI, D.M.F. *et al.* The anticlastogenicity of β-carotene evaluated on human hepatoma cells. *Mutat. Res.*, Amsterdam, v. 303, p. 151-156, 1993.

SALVADORI, D.M.F. *et al.* Effect of β-carotene on clastogenic effects of mitomycin c, methyl methanesulfonate and bleomycin in chinese hamster ovary cells. *Mutagenesis*, Oxford, v. 9, p. 53-57, 1994.

SALVADORI, D.M.F. *et al*. Radioprotection of β-carotene evaluated on mouse somatic and germ cells. *Mutat. Res.*, Amsterdam, v. 356, p. 163-170, 1996.

SANTANA, H. Estudo psicofarmacológico de uma preparação popular reputada como anticonvulsivante. In: SIMPÓSIO DE PLANTAS MEDICINAIS, 8, 1984. Anais… Manaus, 1984, p. 53.

SASAKI, Y.F. *et al*. The clastogen-suppressing effects of green tea, po-lei tea and rooibos tea in cho cells and mice. *Mutat. Res.*, Amsterdam, v. 286, p. 221-232, 1993.

SHIRAKI, M. *et al*. Antioxidative and antimutagenic effects of the theaflavins from black tea. *Mutat. Res.*, Amsterdam, v. 323, p. 29-34, 1994.

SILVA-NETTO, C.R. *et al*. Efeitos antagônicos de plantas medicinais. In: REUNIÃO ANUAL SOBRE EVOLUÇÃO, SISTEMÁTICA E ECOLOGIA MICROMOLECULARES, 9, 1987. Anais... Ribeirão Preto, 1987. p. 15.

STAMPFER, M.J.; RIMM, E.B. A review of the epidemiology of dietary antioxidants and risk of coronary artery disease. *Can. J. Cardiol.*, Dakville, v. 9, p. 14-18, 1993.

STAVRIC, B. *et al*. The effect of teas on the *in vitro* mutagenic potential of heterocyclic aromatic amines. *Food Chem. Toxicol.*, Kidlington, v. 34, p. 515-523, 1996.

STENSVOLD, I. *et al*. Tea consumption, relationship to cholesterol, blood pressure, and coronary and total mortality. *Prev. Med.*, Orlando, v. 21, p. 546-553, 1992.

STICH, H.F. Teas and tea components as inhibitors of carcinogen formation in model systems and man. *Prev. Med.*, Orlando, v. 21, p. 377-384, 1992.

VINITKETKUMNUEN, U. *et al*. Antimutagenicity of lemon grass (*Cymbopogon citratus* Stapf) to various known mutagens in salmonella mutation assay. *Mutat. Res.*, Amsterdam, v. 341, p. 71-75, 1994.

WANG, Z.-Y. *et al*. Protection against polycyclic aromatic hydrocarbon-induced skin tumor initiation in mice by green tea polyphenols. *Carcinogenesis*, Oxford, v. 10, p. 411-415, 1989.

WANG, Z.-Y. *et al*. Protection against ultraviolet B radiation-induced photocarcinogenesis in hairless mice by green tea polyphenols. *Carcinogenesis*, Oxford, v. 12, p. 1527-1530, 1991.

WANG, Z.-Y. *et al*. Protection against Benzo[*a*]pyrene- and N-nitrosodiethylamine-induced Lung and Forestomach Tumorigenesis in A/J Mice by water extracts of green tea and licorice. *Carcinogenesis*, Oxford, v. 13, p. 1491-1494, 1992.

YAMADA, J.; TOMITA, Y. Antimutagenic activity of water extracts of black tea and oolong tea. *Biosci. Biotechnol. Biochem.*, Tokyo, v. 58, p. 2197-2200, 1994.

YAMANE, T. *et al*. Inhibition of Azoxymethane-induced colon carcinogenesis in rat by green tea polyphenol fraction. *Jpn. J. Cancer Res.*, Tokyo, v. 82, p. 1336-1409, 1991.

YANG, S.C.; WANG, Z.-Y. Tea and cancer. *J. Natl. Cancer Inst.*, Bethesda, v. 85, p. 1038-1049, 1993.

YEN, G.-C.; CHEN, H.-Y. Comparison of antimutagenic effect of various tea extracts (Green, Oolong, Pouchong and Black tea). *J. Food Prot.*, Des Moines, v. 57, p. 54-58, 1994.

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